Annals of Clinical and Analytical Medicine
Original Research

Comparison of PCR-negative patients with CT findings and PCR-positive COVID-19 patients

The role of CT in Covid-19

Adem Durmaz[1], Duru Mıstanoğlu Özatağ[2]
[1] Kütahya Yıldırım Beyazıt Family Health Center
[2] Department of İnfectious Diseases, Kütahya Health Sciences University, Kütahya, Turkey

Abstract
Aim: The gold standard diagnostic method for the diagnosis of COVID-19 is based on the demonstration of viral RNA in samples taken from the upper respiratory tract in reverse transcriptase-polymerase chain reaction (RT-PCR). However, in emergencies, the World Health Organization (WHO) also recommends to use computed tomography (CT) in order to reduce the loss of time and to provide rapid diagnosis, treatment and isolation of suspicious cases. In our study, we aimed to compare the laboratory values of patients with PCR negative CT findings and PCR positive patients.
Material and Methods: The medical records of 1280 COVID-19 patients registered at our Family Medicine Center were reviewed retrospectively.
Results: In our study, it was found that 66,70 % of PCR- negative patients with CT findings were aged 60 years and older, and 50.70% of PCR-positive COVID-19 patients were between the ages of 40-59 years; 61.30% of the patients with CT findings and 48% of the PCR-positive patients were male; 73% of PCR-positive patients had lung involvement. When CRP, fibrinogen and D-dimer values were examined, it was found that in PCR-negative COVID-19 patients with CT findings these values were statistically significantly higher.
Discussion: Although the definitive diagnosis of the disease is made using a PCR test, it should not be overlooked that the patients may remain PCR negative, and it should not be forgotten that thoracic tomography findings are a good diagnostic method for this group.

Keywords
Computed Tomography, PCR, COVID-19

DOI: 10.4328/ACAM.20826 Received: 2021-08-20 Accepted: 2021-10-05 Published Online: 2021-10-12 Printed: 2022-01-01 Ann Clin Anal Med 2022;13(1):25-29
Corresponding Author: Duru Mıstanoğlu Özatağ, Kütahya Health Sciences University, Department of İnfectious Diseases, Kütahya, Turkey.
E-mail: durumistan@gmail.com P: +90 505 854 27 90
Corresponding Author ORCID ID: https://orcid.org/0000-0002-0005-192X

Introduction

We are experiencing one of the biggest epidemics in the history of the world. The epidemic that started in China in 2019 and spread all over the world has caused the death of more than 3,9 million people (available at: https://covid19.who.int/ World Health Organization. WHO Coronavirus (COVID-19) Dashboard). The COVID-19 outbreak threatens all humanity, health systems and economies [1]. COVID-19 disease is caused by SARS-COV 2, an RNA virus [2-4]. It can present with a wide clinical spectrum from asymptomatic infection to multiorgan insufficiency. The most common symptoms in patients are fever, weakness, nasal congestion, muscle and joint pain, dry cough, nausea, vomiting, diarrhea, headache, dizziness, smell and taste disturbances [5]. It is also predicted that the epidemic will continue its effect in waves for a while. Due to the emerging new mutations, the effect of vaccines and treatments may decrease [6].

The gold standard diagnostic method for the diagnosis of COVID-19 is based on the demonstration of viral RNA in samples taken from the upper respiratory tract in reverse transcriptase-polymerase chain reaction (RT-PCR) (PCR) [7]. However, in emergencies, the World Health Organization (WHO) also recommends to use computed tomography in order to reduce the loss of time and to provide rapid diagnosis, treatment and isolation of suspicious cases (available at: https://apps.who.int/iris/handle/10665/332326 World Health Organization. Use of chest imaging in COVID-19: a rapid advice guide: web annex A: imaging for COVID-19: a rapid review). In addition, lung imaging methods, in general, help us in cases where the PCR test is initially negative in cases where laboratory tests are not available, especially when RT-PCR is not available, the results are delayed or in the presence of symptoms that make the diagnosis of COVID-19 [8]. Currently, in most countries, diagnosis and treatment planning is made with the findings of thoracic tomography in the presence of emergency kit insufficiency and similar conditions. In COVID-19, the most common image (so-called typical) is ground glass image (74.20%) and consolidation (60.20%) observed in the lower lobes and symmetrically, in addition, reticular appearance, Crazy paving pattern (thickened interlobular septa and intralobular lines observed with ground glass overlapping), air bronchogram, vascular dilatation, interlobular septal thickening, bronchodilation, pleural thickening and pleural effusion, fibrous bands may be observed [9,10]. In our study, we aimed to compare laboratory values of patients with PCR-negative CT findings and PCR-positive patients, and to emphasize the importance of thorax tomography in the diagnosis of the disease with the results obtained.

Material and Methods

The necessary permission for this study was obtained from the Ministry of Health. In addition, on 16.12.2020, the approval was obtained from the Non-Interventional Ethics Committee of Kütahya Health Sciences University with the number 2020/17-23. The medical records of 1280 patients under family medicine follow-up were reviewed retrospectively. Seventy-five patients were included in the study, with at least two negative RT-PCR tests and findings that suspect COVID-19 in their thorax CT (viral pneumonic infiltration, especially ground glass appearance, etc.). In addition, 75 patients with positive RT-PCR test were selected as a control group. Patients whose PCR test was performed only once were excluded from the study. In addition, those who had diseases (such as leukemia, deep vein thrombosis) or those using drugs (anticoagulants, drugs that will affect thrombocytes) were excluded from the study.

The following parameters were evaluated in 150 patients at the time of diagnosis: D-dimer, fibrinogen, C-reactive protein (CRP), hemogram, White blood cell (WBC) count, lymphocytes (lymph), neutrophils (neutral), eosinophils (eos), platelets (PLT) Mean platelet volume (MPV), Platelet distribution width (PDW). RT-PCR (PCR) (Real-Time Polymerase Chain Reaction-Polymerase Chain Reaction) was evaluated using a Bio Rad CFX96 Real-Time PCR machine. The data were recorded in the SSPS (Statistical Package for the Social Science, Inc.; Chicago, IL, USA) 23 package program and statistical analysis was performed. Numerical variables were shown as mean ± standard deviation and median (minimum, maximum), categorical variables as number (n) and percentage (%). Student's t-test and one way ANOVA test were used to compare the categorical variables of the patient and control groups. A $p<0.05$ was considered statistically significant.

Results

In our study, it was found that 66.70% of thePCR-negative patients were 60 years old and above, and 50.70% of the PCR- positive COVID-19 patients were between the ages of 40-59 years; 61.30% of PCR negative patients and 48% of PCR-positive patients were male. While 56% of PCR-negative patients had chronic disease, this rate was 4% in PCR-positive patients (Table 1). When we evaluated the lung tomography findings, 66.70% of PCR-positive COVID-19 patients had typical (ground glass view) viral pneumonic infiltrates, 6.7% had atypical findings and 26.70% (n: 20) of them had no lung involvement.

When CRP, fibrinogen and D-dimer values were examined, it was found that in PCR- negative COVID-19 patients these values were statistically significantly higher than in PCR-positive COVID-19 patients ($p<0.05$). The decrease in WBC values was more prominent in the PCR-positive group (32%). In PCR-negative patients, neutrophil and MPV values were higher, and lymphocyte values were lower ($p<0.05$). In addition, no

Table 1. Basic characteristics of the patients

	PCR negative Patients with CT findings		PCR positive patients
Age	Frequency	Percent	Frequency
20 & under	2	2,7	1
21-39	9	12	21
40-59	14	18,7	38
60 & over	50	66,7	15
Gender			
Male	46	61,3	36
Female	29	38,7	39
Chronic Disease			
Yes	42	56	3
No	33	44	72

significant difference was found between the eosinophil and PLT values in both groups (p> 0.05) (Table 2,3).

Table 2. Laboratory findings of the COVID-19 patients

	PCR negative Patients with CT findings		PCR positive patients	
	Frequency	Percent	Frequency	Percent
CRP				
Normal	6	8	28	37,3
High	69	92	47	62,7
Fibrinogen				
Normal	13	17,3	28	37,3
High	52	69,3	47	62,7
Un-worked	10	13,3	0	0
D-dimer				
Low	0	0	1	1,3
Normal	22	29,3	51	68
High	53	70,7	23	30,7
WBC				
Low	10	13,3	24	32
Normal	56	74,7	49	65,3
High	9	12	2	2,7
Neutrophil				
Low	0	0	0	0
Normal	45	60	66	88
High	30	40	9	12
Lymphocyte				
Low	18	24	3	4
Normal	55	73,3	72	96
High	2	2,7	0	0
Eosinophil				
Low	66	88	71	94,7
Normal	6	8	4	5,3
High	3	4	-	-
Platelet				
Low	3	4	3	8
Normal	67	89,3	67	88
High	5	6,7	5	4
MPV				
Low	0	0	0	0
Normal	70	93,3	75	100
High	5	6,7	0	0
PDW				
Low	0	0	0	0
Normal	75	100	75	100
High	0	0	0	0

Table 3. Evaluation of Blood Values of CT Positive Patients and PCR Positive Patients

	Group	n	Mean	SD	t	p
CRP						
	CT Positive	75	2,6267	,48695	10,961	,000
	PCR Positive	75	1,9200	,27312	10,961	,000
Fibrinogen						
	CT Positive	65	2,6267	,48695	10,840	,000
	PCR Positive	75	1,8000	,40311	10,840	,000
D-dimer						
	CT Positive	75	2,2933	,48695	7,597	,000
	PCR Positive	75	1,7067	,45836	7,597	,000
WBC						
	CT Positive	75	1,7067	,51395	-3,360	,001
	PCR Positive	75	1,9867	,50653	-3,360	,001
Neutrophil						
	CT Positive	75	2,1200	,32715	-4,097	,000
	PCR Positive	75	2,4000	,49320	-4,097	,000
Lymphocyte						
	CT Positive	75	1,9600	,19728	2,927	,004
	PCR Positive	75	1,7867	,47344	2,927	,004
Eosinophil						
	CT Positive	75	1,0533	,22621	-1,783	,077
	PCR Positive	75	1,1600	,46615	-1,783	,077
Platelet						
	CT Positive	75	2,0267	,32770	1,211	,228
	PCR Positive	75	1,9600	,34641	1,211	,228
MPV						
	CT Positive	75	2,0000	,00000	-3,360	,000
	PCR Positive	75	2,0667	,25112	-3,360	,000

Discussion

In thoracic tomography, a ground glass image may occur due to the presence of a small amount of fluid or cells in the alveoli, or a thickening of the wall in the alveoli. COVID-19 is one of the most common images, but it can also be observed in pulmonary edema, acute respiratory distress syndrome (ARDS), lung malignancies, idiopathic pulmonary fibrosis, interstitial lung diseases (such as pneumonia, silicosis, hypersensitivity pneumonia, and sarcoidosis) (Figures 1-2) [11,12].

During the pandemic process, the most important principles in our fight against COVID-19 are early diagnosis and initiation of appropriate treatment of suspected patients, and isolation and vaccination studies of cases that have come into contact with these patients. In order to avoid wasting time in emergencies, WHO recommends that pre-diagnosis with thoracic CT findings and subsequent confirmation of the diagnosis with RT-PCR is one of the most frequently used methods. For this reason, in most countries, in the presence of a suspicious (especially ground glass view) image on thoracic CT scan, COVID-19 is diagnosed, treatment is started until blood tests are taken and the patient is isolated. Generally, it has been observed that the PCR test becomes positive within an average of 2-8 days from the onset of the disease [13]. In a study conducted by Tanyeri, CT specificity was found to be 69% for COVID-19, and it was reported that, in suspicious cases, it would be more rational to perform a CT scan first [14]. Even if the PCR test is negative due to reasons (such as laboratory error or insufficient viral material in the sample), the person is considered a COVID-19 patient and his isolation continues with treatment. In the study conducted by Vannucci et al., the most accurate sampling method reported as broncho-alveolar lavage (BAL). However, it is not a highly preferred method due to the risks during BAL (especially the risks of contamination and complications)[15]. In our study, we found that in PCR-negative COVID-19 patients with CT findings, some of the patients were negative despite repeating the PCR test two or three times. In our study, we found that the mean age of the PCR-negative group with CT findings was higher than that of the PCR-positive group, and that CRP, Fibrinogen

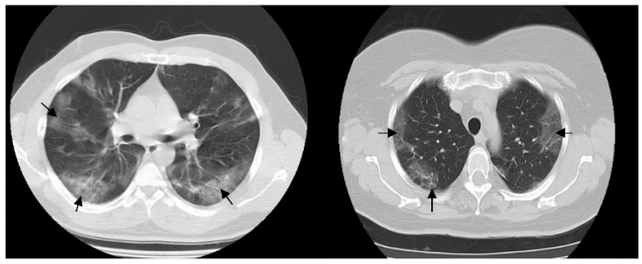

Figure 1. Ground-glass densities in both lungs with a tendency to coalesce, being more intense in the peripherally located basals

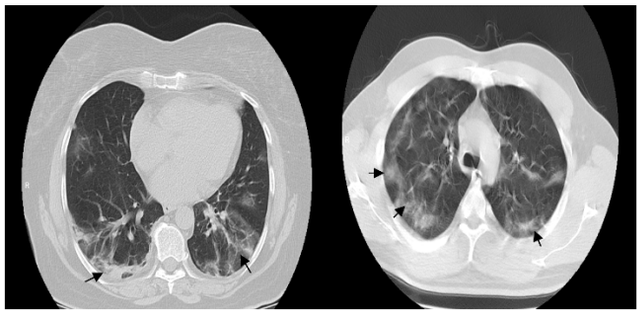

Figure 2. Subpleural scattered ground-glass densities in the lower lobes of both lungs

and D-Dimer values were also higher, indicating that the risk exacerbation of the disease increases with advanced age.

In another study by Ai et al., PCR positivity was found in 68.80% of patients with suspected COVID-19, and lung involvement on CT was found in 88% of these suspicious patients. In addition, they found involvement in thorax CT in 97% of patients with a positive PCR result [16]. In our study, we found involvement in CT in 73% of the patients who were PCR positive. Chen et al. reported that, despite multiple tests for COVID-19, viral RNA could be shown only in the fifth PCR test in a patient with ground glass in the lungs [17].

In studies conducted, high CRP was detected in 60% of COVID-19 patients, and it was reported that it may increase in severe viral infection, viremia and viral sepsis [18,19]. In our study, CRP values were found to be increased in 92% of PCR-negative patients with CT findings and in 62.70% of PCR-positive patients ($p<0.05$). It has been observed in studies that D-dimer and fibrinogen values are increased in COVID-19 patients. This is considered a manifestation of disseminated coagulopathy and is considered to inform us about the severity and prevalence of COVID-19 [20]. In our study, in PCR-negative patients with CT findings, D-dimer values were significantly higher than in PCR- positive patients (1121.88 ng/mL vs 618.2 ng/mL, $p <0.05$). In negative patients, it was significantly higher than PCR positive (499.6 mg/dl vs 390.1 mg/dl, $p <0.05$). These findings show that COVID-19 disease is more common and more severe due to lung involvement.

Lymphopenia (absolute lymphocyte count is defined as <1.0×103/μL) is a frequently expected finding for COVID-19 infection and is observed in cases where the immunological response to the virus is reduced [19]. In our study, lymphopenia was more common in PCR-negative patients with CT findings (1.61×103/uL vs 1.72×103/uL, $p <0.05$). In a study conducted by Huang et al. on 41 patients, the rate of lymphopenia was found to be 63%, lung involvement was demonstrated by CT in all of these patients [21], and it was observed that this rate increased to 83% in patients requiring hospitalization [18]. Similarly, lymphopenia was observed more frequently in PCR-negative patients with CT findings in our study. This can be explained by the fact that the infection progressed more clinically. Lippi et al. reported in their study that more lymphopenia developed in patients who died due to COVID-19. Lymphopenia, CRP, D-dimer, prothrombin time, increases in troponin and creatine phosphokinase are considered to be poor prognostic factors [18].

In COVID-19 infection, the neutrophil count was found to be higher in PCR-negative patients with CT findings compared to PCR-positive patients (6.16×103/uL vs 4.08×103/uL, $p<0.05$). Studies have found that neutrophil counts increase in cases of bacterial superinfection [18]. In addition, MPV values used as one of the inflammatory markers are higher in PCR-negative patients with CT findings compared to PCR- positive patients (9.70/L vs 9.48/L, $p <0.05$). In a study conducted by Güçlü et al. on COVID-19 patients, it was found that the MPV values were high in these patients, and they even showed that an increase of 1 unit in MPV increased mortality by 1.76 times [22]. There are publications reporting that low eosinophil values may be observed in patients with COVID-19, and if these low values persist, the prognosis may be adversely affected [23]. In our study, we observed decreases in eosinophil counts in both groups, but we found that there was no significant difference in eosinophil counts between PCR-negative patients with CT findings and PCR-positive patients (0.106×103/ uL vs 0.063×103/uL, $p> 0.05$).

In our study, no significant difference was found in PDW values between PCR-negative patients with CT findings and PCR-positive patients (16.17 ratio vs 16.08 ratio, $p> 0.05$). Likewise, no significant difference was found between platelet counts (237.80 ×103/uL vs. 228.10×103/uL, $p>0.05$). It has been reported that thrombocytopenia may occur in severe COVID-19 infection [20]. In our study, platelet counts were within normal limits.

Conclusion

In our study, we found a high rate of deterioration of inflammatory signs and values related to coagulopathy in our PCR-negative patients with CT findings. This is due to the fact that COVID-19 disease has progressed clinically due to lung involvement in this group due to the diagnosis of CT involvement in all of our PCR-negative patient groups. Although the definitive diagnosis of the disease is made with the PCR test, it should not be overlooked that patients may remain PCR negative, and it should not be forgotten that thorax tomography findings are a good method for diagnosing this group.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: *None*

Conflict of interest
None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

References
1. Rodriguez-Morales AJ, Bonilla-Aldana DK, Balbin-Ramon GJ, Rabaan AA, Sah R, Paniz-Mondolfi A, et al. History is repeating itself: Probable zoonotic spillover as the cause of the 2019 novel Coronavirus Epidemic. Infez Med. 2020;28(1):3-5.
2. Asselah T, Durantel D, Pasmant E, Lau G, Schinazi RF. COVID-19: Discovery, diagnostics and drug development. J Hepatol. 2021;74(1):168-84.
3. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nature Microbiology. 2020;5(4):536-44.
4. Gralinski LE, Menachery VD. Return of the Coronavirus: 2019-nCoV. Viruses. 2020;12(135):1-8.
5. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.
6. Chen J, Wang R, Wang M, Wei GW. Mutations Strengthened SARS-CoV-2 Infectivity. J Mol Biol. 2020;432(19):5212-26.
7. Feng H, Liu Y, Lv M, Zhong J. A case report of COVID-19 with false negative RT-PCR test: necessity of chest CT. Jpn J Radiol. 2020;38(5):409-10.
8. Akl EA, Blažić I, Yaacoub S, Frija G, Chou R, Appiah JA, et al. Use of Chest Imaging in the Diagnosis and Management of COVID-19: A WHO Rapid Advice Guide. Radiology. 2021;298(2):E63-9.
9. Wang J, Xu Z, Wang J, Feng R, An Y , Ao W, et al. CT characteristics of patients infected with 2019 novel coronavirus: association with clinical type. Clin Radiol. 2020;75(6):408-14.
10. Ye Z, Zhang Y, Wang Y, Huang Z, Song B. Chest CT manifestations of new coronavirus disease 2019 (COVID-19): a pictorial review. Eur Radiol. 2020;30(8):4381-9.
11. Hansell DM, Bankier AA, MacMahon H, McLoud TC, Müller N, Remy J. Fleischner Society: Glossary of Terms for Thoracic Imaging. Radiology. 2008;246(3):697–722.
12. Xu X, Yu C, Qu J, Zhang L, Jang S, Huang D, et al. Imaging and clinical features of patients with 2019 novel coronavirus SARS-CoV-2. Eur J Nucl Med Mol Imaging. 2020;47(5):1275-80.
13. Xie X, Zhong Z, Zhao W, Zheng C, Wang F, Liu J. Chest CT for Typical 2019-nCoV Pneumonia: Relationship to Negative RT-PCR Testing. Radiology. 2020;296(2):E41-5.
14. Tanyeri A. RT-PCR Results and Chest CT Imaging Features in Patients Hospitalized to Yozgat City Hospital with COVID-19 Suspicion. Ankara Med J. 2021;(1):99-114.
15. Vannucci J, Ruberto F, Diso D, Galardo G, Mastroianni CM, Raponi G, et al. Usefulness of bronchoalveolar lavage in suspect COVID-19 repeatedly negative swab test and interstitial lung disease. J Glob Antimicrob Resist. 2020;23:67-9.
16. Ai T, Yang Z, Hou H, Zhan C, Chan C, Lv W, et al. Correlation of Chest CT and RT-PCR Testing for Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. Radiology. 2020;296(2):E32-E40.
17. Chen LD, Li H, Ye YM, Wu Z, Huang YP, Zhang WL, et al. A COVID-19 patient with multiple negative results for PCR assays outside Wuhan, China: a case report. BMC Infect Dis. 2020;20(517):1-4.
18. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. JAMA. 2020;324(8):782-93.
19. Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. Clin Chem Lab Med. 2020;58(7):1131-4.
20. Han H, Yang L, Liu R, Liu F, Wu KL, Li J, et al. Prominent changes in blood coagulation of patients with SARS-CoV-2-infection. Clin Chem Lab Med. 2020;58:1116–20.
21. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.
22. Güçlü E, Kocayiğit H, Okan HD, Erkorkmaz U, Yürümez Y, Yaylacı S, et al. (2020). Effect of COVID-19 on platelet count and its indices. Rev Assoc Med Bras. 2020;66(8):1122-7.
23. Rosenberg HF, Foster PS. Eosinophils and COVID-19: diagnosis, prognosis, and vaccination strategies. Semin Immunopathol. 2021;43:383-92.

How to cite this article:
Adem Durmaz, Duru Mıstanoğlu Özatağ. *Comparison of PCR-negative patients with CT findings and PCR-positive COVID-19 patients. Ann Clin Anal Med 2022;13(1):25-29*